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Evaluation of Modifications to Extraction Procedures Used in Analysis of Environmental Samples from Superfund Sites

CORNELIUS A. VALKENBURG and WILLIAM D. MUNSLOW
Lockheed Engineering and Sciences Co., Inc., 1050 E Flamingo Rd, Suite 120, Las Vegas, NV 89119
LARRY C. BUTLER
U.S. Environmental Protection Agency, PO Box 93478, Las Vegas, NV 89193-3478

Recoveries from an aqueous sample of the semi-volatile analytes listed on the EPA Target Compound List (TCL) are compared using 6 different methylene chloride extraction procedures. Four experimental designs incorporating a continuous extraction apparatus are evaluated, and 2 experimental designs using separatory funnel methods are tested. In addition, 2 concentration procedures are compared, and the loss of analytes associated with both extraction and concentration procedures are determined. These studies indicate that the most efficient and economical technique for the extraction of these compounds from an aqueous matrix is a single continuous extraction procedure performed at pH 2.

The Contract Laboratory Program (CLP) is administered by the U.S. Environmental Protection Agency (EPA) under the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA, Superfund) as amended in 1986 as the Superfund Amendments and Reauthorization Act (SARA) (1). CLP is a nationwide network of independent laboratories under contract to EPA which are available to perform analyses on environmental samples taken to support remedial decisions and regulatory activities. The procedures for CLP analyses are explicitly described in the Statement-of-Work (SOW) for Organics Analysis: Multi-Media Multi-Concentration (2). SOW contains the procedures for the analysis of specific organic compounds which are listed on the Superfund Target Compound List (TCL) of SOW.

Analytical procedures in SOW for these compounds are not designed to achieve the maximum recovery of any one class of compound. Instead, they were designed as an efficient, economical approach for simultaneous determination of a large number of analytes. For aqueous samples, SOW procedures can be subdivided into 3 classes based on the analytical methodology. (*Note*: Only procedures for extraction of aqueous samples and procedures for concentration of the extracts are discussed in this paper.) Volatile compounds on the Target Compound List are analyzed by purge and trap gas chromatography/mass spectrometry (GC/MS); halogenated pesticides are extracted from aqueous samples by a single liquid-liquid extraction at a moderate pH (5–9) and quantified by gas chromatography with electron-capture detection (GC/EC); and the remainder of the TCL analytes (termed semi-volatiles) are extracted from aqueous samples by methylene chloride liquid-liquid extraction and quantified by GC/MS. Extraction of semi-volatile TCL components into methylene chloride requires making the aqueous matrix basic to a pH >11, extracting with methylene chloride, acidifying the aqueous phase to a pH <2, and then re-extracting with methylene chloride. The methylene chloride extracts from each pH extraction are concentrated separately and combined just prior to analysis by GC/MS. SOW allows the extraction to be done either by separatory funnel or by continuous liquid-liquid extraction. The latter is recommended for samples which form emulsions or are otherwise difficult to extract by the separatory funnel procedure.

The principles of trace organic analysis have been described (3) and a large variety of methods and apparatus for sample preparation have been evaluated by the analytical chemistry community (4–12). The procedures as used in the Contract Laboratory Program for the analysis of semi-volatiles were adapted from Method 625 (13) and these procedures have been the subject of continuous study and modification. The development and evaluation of the procedures required by SOW have been reviewed (14–16). Originally, separate GC/MS analyses of each of the 2 pH extracts were performed on packed columns appropriate for the compounds extracted. Also, prior to the introduction of capillary GC columns, other workers tested procedures wherein the extracts from environmental matrixes were separated into several fractions by wet chemical techniques to achieve partial separation of the compounds on the basis of molecular structure (17, 18). This greatly simplified the chromatography required in the final quantification step. Eventually, the use of fused-silica capillary columns was applied to the ex-

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Table 1. Average recovery of all analytes in each extraction experiment

Cmpd ID	Compound name	Amount spiked, µg/L	Exp. A		Exp. B		Exp. C		Exp. D		Exp. E		Exp. F	
			Av. % rec.	RSD, %	Av. % rec.	RSD, %	Av. % rec.	RSD, %	Av. % rec.	RSD, %	Av. % rec.	RSD, %	Av. % rec.	RSD, %
1	Dimethylphthalate	80	21.4	3.4	15.6	3.7	104.5	0.2	99.8	4.1	80.8	3.3	102.8	1.9
2	*N*-Nitroso-di-*n*-propylamine	80	75.1	2.1	65.6	3.8	120.1	21.1	96.5	4.0	101.6	5.4	93.2	2.0
3	bis(2-Chloroisopropyl)ether	80	82.6	3.8	79.0	3.4	92.2	8.0	81.9	14.5	93.5	1.3	83.0	2.9
4	1,2,4-Trichlorobenzene	80	62.4	4.2	57.0	9.4	83.0	3.1	59.3	25.6	75.9	1.9	71.2	2.1
5	Diethylphthalate	80	58.8	2.6	50.0	1.0	103.1	1.0	100.0	4.3	104.8	1.0	106.5	4.8
6	bis(2-Chloroethyl)ether	80	88.8	1.6	87.2	2.2	97.6	7.9	96.6	5.3	95.7	1.0	90.6	3.5
7	bis(2-Chloroethoxy)methane	80	86.3	2.4	85.5	2.0	98.7	3.9	95.6	4.1	92.1	1.6	88.4	3.0
8	Di-*n*-octylphthalate	80	85.4	2.7	83.5	7.6	92.8	3.3	105.4	12.6	95.1	1.4	85.9	2.1
9	4-Bromophenyl-phenylether	80	97.5	6.3	91.1	6.9	109.8	2.5	108.1	4.1	99.8	2.9	100.3	2.0
10	Butylbenzylphthalate	80	93.6	6.3	92.7	8.3	105.1	0.9	108.7	9.8	99.8	2.6	98.5	2.6
11	4-Chlorophenyl-phenylether	80	98.3	3.1	85.9	3.5	107.0	1.3	106.7	4.5	104.8	0.5	103.0	0.8
12	Di-*n*-butylphthalate	80	98.0	6.7	91.6	0.6	107.2	1.3	107.0	5.5	106.4	3.3	102.5	3.8
13	bis(2-Ethylhexyl)phthalate	80	117.1	4.3	105.7	5.0	124.4	1.6	115.0	12.5	191.1	7.4	188.3	13.7
14	Hexachlorocyclopentadiene	80	1.7	1.3	0.4	0.5	52.4	1.5	33.1	17.1	54.7	3.5	59.7	4.5
15	Hexachloroethane	80	22.8	4.0	20.6	3.7	56.1	1.3	42.9	9.3	57.1	0.9	53.5	1.7
16	1,3-Dichlorobenzene	80	33.0	3.1	29.2	3.8	61.0	1.4	41.7	21.0	69.6	1.2	65.5	2.1
17	1,4-Dichlorobenzene	80	34.7	3.5	30.1	4.1	61.7	1.2	42.7	21.3	70.6	1.0	66.1	2.2
18	1,2-Dichlorobenzene	80	37.7	3.2	32.9	4.9	65.3	1.7	45.2	22.1	74.1	1.1	69.2	2.2
19	Hexachlorobutadiene	80	30.0	5.1	25.8	7.1	81.8	3.4	61.2	18.5	65.3	1.9	62.6	1.9
20	Isophorone	80	93.7	4.1	94.8	2.1	59.5	1.1	57.4	2.6	98.6	1.4	96.1	2.5
21	Nitrobenzene	80	86.6	4.0	85.6	0.7	99.0	4.2	94.0	8.9	93.4	2.0	90.7	5.1
22	2-Methylnaphthalene	80	69.7	4.5	60.0	15.0	98.0	2.4	84.8	15.0	92.8	1.6	89.0	2.1
23	2-Chloronaphthalene	88	70.0	4.1	61.4	10.2	91.3	1.7	85.3	7.1	80.7	2.0	80.8	3.4
24	2,6-Dinitrotoluene	80	97.8	7.1	96.4	1.6	102.6	1.1	99.7	3.0	105.9	2.4	102.3	2.6
25	2,4-Dinitrotoluene	80	100.8	8.5	98.2	3.3	100.9	1.4	98.6	5.2	109.0	4.2	103.5	5.2
26	Dibenzofuran	80	90.4	5.0	81.4	3.4	103.2	0.0	101.2	2.9	97.3	1.8	97.2	2.5
27	Hexachlorobenzene	80	95.3	5.7	88.9	4.4	106.5	2.2	106.7	3.6	99.6	2.9	98.6	1.1
28	Benzyl alcohol	80	91.1	3.0	91.7	1.9	99.6	3.9	99.9	4.6	84.8	1.3	80.0	2.6
29	Naphthalene	80	61.6	2.1	52.1	13.5	90.2	4.2	68.7	22.5	89.0	1.1	84.8	2.7
30	Benzo(b)fluoranthene	80	88.2	5.2	82.1	6.3	101.4	2.4	108.9	8.6	97.3	0.7	94.8	4.7
31	Dibenz(a,h)anthracene	80	92.7	2.9	91.5	2.5	97.5	3.5	97.6	10.4	82.5	2.2	84.8	3.2
32	Acenaphthene	80	88.5	4.5	80.3	5.5	102.3	2.9	98.0	2.8	93.6	1.2	92.7	1.9
33	Acenaphthylene	80	90.4	3.8	78.9	6.5	101.8	2.3	96.9	2.5	94.3	1.4	94.4	3.0
34	Benzo(a)pyrene	80	85.3	2.9	83.4	5.8	89.0	0.6	91.8	8.0	87.0	0.3	86.4	1.7
35	Indeno(1,2,3-cd)pyrene	80	97.0	5.1	91.4	3.9	96.7	4.2	95.7	12.3	79.2	7.8	82.9	3.8
36	Fluorene	80	99.0	3.1	87.8	2.6	105.6	1.0	103.5	3.9	103.4	0.9	101.1	0.8
37	Benzo(g,h,i)perylene	80	94.8	1.8	93.3	2.9	99.4	2.1	98.8	8.8	80.8	1.8	84.5	2.9
38	Fluoranthene	80	100.7	9.1	92.9	2.5	107.1	0.4	106.5	6.3	104.0	4.6	98.8	2.4
39	Phenanthrene	80	98.2	5.5	91.4	1.4	110.8	0.9	106.8	1.8	105.5	1.7	102.4	1.5
40	Benz(a)anthracene	80	103.1	7.7	95.2	4.8	109.6	0.5	109.0	6.1	101.4	2.5	99.3	2.6
41	Pyrene	80	96.1	7.4	90.5	6.5	103.5	2.1	109.9	6.8	101.6	3.3	98.1	2.3
42	Anthracene	80	99.8	2.4	83.6	4.2	102.9	0.7	102.3	2.2	100.6	2.3	100.1	1.8
43	Chrysene	80	98.0	5.5	92.6	4.2	106.4	0.5	106.9	6.3	98.2	0.7	96.9	1.5
44	Benzo(k)fluoranthene	80	91.5	3.4	83.6	2.1	106.8	15.1	102.4	11.7	85.3	8.7	89.9	2.5

tractable priority pollutants, allowing both the acidic and base/neutral fractions to be analyzed on the same column (19–23). This modification reduced the GC/MS time required for these analyses, prevented analytical problems caused by incomplete separation of acidic and base/neutral compounds,and resulted in a significant cost savings to EPA.

In 1986, Slayton and Trovato (24) investigated the effect of extracting semi-volatile compounds after first acidifying the aqueous matrix, making the solution basic, and then re-extracting. In addition, they compared the recoveries obtained by continuous extraction with those obtained by the separatory funnel technique. The Slayton-Trovato study was conducted on a limited number of the TCL analytes and indicated that an acid-neutral continuous extraction sequence was effective and labor saving.

The present paper reports our results for a variety of simplifying modifications to the liquid-liquid continuous extraction procedure, and compares these results to analogous extractions uing the separatory funnel extraction procedure. In addition, the extent of analyte loss during the concentration steps of the analytical procedure was evaluated.

Experimental

Preparation of Spiking Solutions

The compounds used to fortify the aqueous matrix were contained in 3 spiking solutions. Solution 1 contained compounds No. 66–80 (Table 1) and was prepared from the EPA's Quality Assurance Materials Bank (QAMB) multicomponent standard No. C090-015. Two mL of this standard was added to a 50 mL amber volumetric flask and diluted to volume with methanol. Solution 2 contained compounds No. 1–65, 81, and 82 (Table 1) and was prepared by combining 2 mL each of QAMB multicomponent standards No. C097-01, C094-01, C098-01, C075, and C093-01 and individual standards No. EC0000610301 and EC0000260103 in a 50 mL amber volumetric flask and diluting to volume with methanol. Solution 3 contained the remaining compounds (No. 83–

Table 1 (continued). Average recovery of all analytes in each extraction experiment

Cmpd ID	Compound name	Amount spiked, µg/L	Exp. A Av. % rec.	Exp. A RSD, %	Exp. B Av. % rec.	Exp. B RSD, %	Exp. C Av. % rec.	Exp. C RSD, %	Exp. D Av. % rec.	Exp. D RSD, %	Exp. E Av. % rec.	Exp. E RSD, %	Exp. F Av. % rec.	Exp. F RSD, %
45	4-Nitroaniline	50	78.0	4.4	48.0	9.7	61.0	7.6	56.0	4.9	45.0	2.5	36.0	3.2
46	3-Nitroaniline	50	68.0	1.7	38.0	3.6	77.0	4.5	60.0	8.5	18.0	2.1	14.0	2.2
47	2-Nitroaniline	50	85.0	2.2	90.0	3.6	81.0	0.8	81.0	1.6	53.0	1.1	51.0	1.9
48	4-Chloroaniline	50	84.0	5.6	55.0	1.9	81.0	0.0	84.0	3.9	41.0	2.2	43.0	1.3
49	Heptachlor	80	95.9	3.7	89.4	3.9	103.2	2.8	103.7	4.6	99.4	3.1	96.6	2.8
50	Heptachlor epoxide	80	101.9	5.8	94.6	0.4	105.3	1.4	105.7	5.5	103.8	5.7	99.6	4.3
51	Methoxychlor	80	98.1	3.3	92.1	5.7	105.2	1.8	108.4	7.1	97.3	4.2	95.6	0.6
52	Endosulfan sulfate	80	92.5	5.4	88.9	5.6	100.2	0.4	104.9	9.5	97.2	2.9	93.3	2.7
53	*p,p'*-DDT	80	95.3	4.8	89.1	7.7	100.6	1.1	107.4	9.1	98.4	4.0	94.1	2.8
54	Endrin	80	98.9	5.4	99.8	14.9	107.3	0.6	114.1	10.4	99.8	7.0	93.9	5.3
55	alpha-BHC	80	81.9	5.0	76.2	5.6	107.0	0.9	105.8	4.8	99.6	3.0	99.6	1.6
56	delta-BHC	80	75.7	5.6	72.4	1.3	107.2	0.2	105.4	5.8	96.7	3.4	98.9	3.6
57	Dieldrin	80	93.3	5.1	89.7	7.6	100.0	0.8	109.2	9.7	101.7	4.5	96.8	3.5
58	gamma-BHC	80	82.8	3.9	74.8	1.7	106.4	1.5	106.0	4.6	100.2	3.2	99.8	2.0
59	Aldrin	80	97.5	4.6	91.9	2.8	106.7	0.7	106.8	5.7	100.9	3.1	98.4	2.9
60	*p,p'*-DDD	80	91.5	4.1	86.6	7.9	99.9	3.5	100.3	3.9	97.9	1.0	98.5	1.4
61	Endrin ketone	40	39.3	11.4	13.0	2.0	32.4	2.7	38.3	3.0	33.6	4.8	90.0	1.7
62	beta-BHC	80	99.7	5.9	95.8	1.5	106.4	0.8	104.7	5.5	101.6	4.5	98.0	5.0
63	*p,p'*-DDE	80	99.2	4.2	94.7	6.8	107.0	1.3	114.4	9.0	106.8	2.5	101.5	2.9
64	alpha-Endosulfan	80	85.9	4.6	86.2	8.3	104.4	1.6	113.2	9.7	100.8	4.6	100.2	3.9
65	beta-Endosulfan	80	93.5	2.8	88.0	3.0	101.9	1.8	102.5	3.5	100.6	1.7	97.1	2.8
66	Benzoic acid	40	53.3	11.1	60.4	4.2	62.6	0.2	59.6	8.5	8.2	2.6	2.7	2.2
67	2,4-Dimethylphenol	40	65.2	6.4	51.4	7.9	87.0	1.6	82.8	5.0	81.2	2.2	77.4	2.1
68	2,4-Dinitrophenol	40	76.6	0.6	93.0	12.1	84.1	4.0	90.9	9.5	87.9	12.5	77.7	6.9
69	4,6-Dinitro-2-methylphenol	40	86.5	3.7	97.6	14.3	96.3	3.1	97.2	4.6	96.2	10.5	89.4	5.0
70	2,Nitrophenol	40	83.7	1.9	86.0	2.6	96.4	5.6	95.5	3.2	88.7	4.3	85.7	4.6
71	4-Methylphenol	40	87.7	1.8	84.0	3.1	95.9	5.6	93.4	2.4	77.0	1.9	71.7	1.3
72	2,4-Dichlorophenol	40	86.3	1.1	86.6	1.6	97.2	6.3	93.7	3.2	90.5	3.4	86.7	2.9
73	2,4,5-Trichlorophenol	40	90.5	2.6	91.0	3.2	102.3	3.4	98.1	2.3	93.4	2.8	91.9	3.2
74	2-Chlorophenol	40	85.9	0.3	84.6	2.6	95.5	7.2	93.5	3.4	88.0	2.8	83.3	3.1
75	4-Nitrophenol	40	97.9	12.5	107.1	1.8	95.2	0.8	92.9	7.1	42.4	3.1	34.4	4.4
76	4-Chloro-3-methylphenol	40	93.8	4.2	94.3	1.3	102.2	1.1	96.8	4.2	99.2	2.6	94.1	1.8
77	Pentachlorophenol	40	94.9	8.7	101.1	4.6	102.8	2.7	99.0	1.9	96.0	5.4	89.2	5.2
78	2-Methylphenol	40	85.8	1.3	82.1	2.3	96.3	5.9	95.4	4.5	82.2	1.8	77.7	2.9
79	2,4,6-Trichlorophenol	40	90.9	2.8	96.7	3.7	104.1	2.6	100.5	3.4	92.2	4.1	92.9	3.5
80	Phenol	40	85.9	0.7	83.7	4.4	94.4	6.6	92.9	3.4	46.7	1.5	43.4	0.8
81	*N*-Nitrosodiphenylamine	50	105.9	1.6	90.9	2.1	71.1	0.6	67.5	5.4	96.7	3.2	108.7	1.1
82	3,3'-Dichlorobenzidine	50	90.3	6.6	106.5	10.7	42.4	21.6	45.7	14.2	74.4	6.3	18.7	1.0
83	2-Fluorophenol	200	83.7	1.2	80.7	5.4	83.4	8.6	78.2	4.6	62.1	2.8	56.8	2.0
84	D5-Phenol	200	93.9	1.2	89.8	5.4	99.9	5.5	99.7	4.0	47.7	0.4	42.5	1.3
85	2-Fluorobiphenyl	100	83.5	3.4	78.0	1.9	86.9	2.7	82.3	7.2	88.4	1.0	86.8	2.7
86	D5-Nitrobenzene	100	84.2	0.4	82.8	2.1	90.4	5.5	90.5	2.1	88.7	1.6	84.9	2.4
87	2,4,6-Tribromophenol	200	96.3	7.0	93.6	10.5	93.6	2.7	88.3	3.3	102.9	3.1	95.4	4.9
88	D14-Terphenyl	100	109.7	5.3	102.8	3.0	116.1	2.1	119.1	5.0	122.9	1.7	115.2	2.1

88). The compounds in solution 3 are used as surrogates during the analysis of environmental samples as described in the Statement-of-Work (2). The spiking solutions were stored at 4°C. Thus, prepared solutions 1, 2, and 3 contained 50–100 ng/mL of each analyte, depending on the concentration of that analyte in the QAMB standard.

Extraction of an Aqueous Matrix

One L of liquid chromatography (LC) grade water was spiked with 1 mL of each of the 3 spiking solutions prior to extraction. The procedures for liquid-liquid extractions using either a separatory funnel or a continuous extractor are described in SOW. The continuous extractors (Ace Glass, 639 S Hancock St, Louisville, KY, Part No. 30604-000) used in this experiment are designed to extract 1 L water with solvents heavier than water. Each extractor was attached to a 1 L boiling flask containing 500 mL methylene chloride. The extractors were refluxed for 18 h with vigorous boiling and used tap water (ca 16°C) for condensor cooling. Modifications to the SOW extraction protocol included reversal of the pH sequence and elimination of the extraction solvent collection and renewal step normally applied when pH of the aqueous matrix is changed. The aqueous matrix was made acidic with 6N H_2SO_4 and was made basic with 10N NaOH. Methylene chloride extracts were concentrated using SOW procedures with the nitrogen "blowdown" concentration option at 35°C for the final concentration. The concentrated extracts were stored in Teflon™-capped vials at 4°C.

Quantification of Analytes

The recovery of all analytes was measured by GC/MS as described in the Statement-of-Work. This method uses internal standards to calculate relative response factors for a selected ion of each TCL compound and thus quantitates from the reconstructed ion chromatogram. The standard solutions used for determining the relative response factors

Table 2. Comparison of selected analyte recoveries (% mean recoveries for triplicate analyses) after concentration by micro-Snyder and nitrogen blowdown

Cmpd ID	Compound name	Micro-Snyder	SD	N_2 blowdown	SD
5	Diethylphthalate	102.9	1.4	105.3	0.7
12	Dibutylphthalate	102.8	1.2	104.7	0.5
15	Hexachloroethane	96.9	3.1	96.1	0.5
17	1,4-Dichlorobenzene	97.0	3.0	99.4	0.7
18	1,2-Dichlorobenzene	89.9	4.8	96.1	0.5
19	Hexachlorobutadiene	99.9	2.6	100.1	0.1
23	2-Chloronaphthalene	103.8	2.4	101.8	0.8
26	Dibenzofuran	105.3	2.2	104.4	1.3
27	Hexachlorobenzene	106.7	2.3	109.7	1.7
29	Naphthalene	92.7	4.1	96.8	0.5
32	Acenaphthalene	104.7	2.3	103.2	1.1
38	Fluoranthrene	106.0	4.5	108.2	1.7
39	Phenanthrene	102.0	1.2	103.9	0.6
41	Pyrene	105.7	4.6	107.9	1.7
43	Chrysene	95.9	8.5	99.7	2.9
45	4-Nitroaniline	106.6	1.3	107.7	0.8
68	2,4-Dinitrophenol	105.1	1.8	104.2	1.6
74	2-Chlorophenol	98.2	3.5	100.0	0.9
75	4-Nitrophenol	104.6	1.2	106.8	0.6
77	Pentachlorophenol	103.6	1.2	107.5	1.5
80	Phenol	92.6	5.7	97.2	0.7
83	2-Fluorophenol	96.0	4.5	99.7	1.9

were obtained from QAMB. Identity of isomers was confirmed by relative retention time. Percent recovery for each analyte was determined by comparing the concentration measured in the extract vs the theoretical concentration spiked into the water. Quantification was not made relative to the spiking solution because of peak splitting in the GC/MS analysis caused by the methanol solvent (24). Analyses were performed on a Finnigan 4023 GC/MS. The GC was equipped with a 30 m, 0.32 μm, DB-5 column (J&W Scientific) and temperature programmed from 30°C to 300°C at 8°/min with an initial hold at 30°C for 4 min. The MS was operated in the EI mode (70 eV, scan time 1.0 s, mass range 45–550 m/z, ion source temperature 270°C). Automated quantification was performed with a DEC computer operating the "Autoquan" procedure of INCOS 3.3 Software. Quantification ions were those described in SOW and all QA/QC criteria specified in SOW were achieved.

Comparison of Concentration Techniques

The recovery of the compounds listed in Table 2 was measured after a standard solution was concentrated to 0.5 mL from 6 mL by the nitrogen blowdown technique and the micro-Snyder technique. A stock solution containing 50 ng/μL of compounds 5, 12, 15, 18, 29, 39, 45, 75, 77, and 80 in methylene chloride was prepared. A 10 mL concentrator tube was charged with 6 mL of the stock solution, boiling chips were added, and a small amount of methylene chloride was added to the top of the column. The sample was then concentrated to an apparent volume of 0.5 mL in a water bath maintained at 90°C. The apparatus was removed from the bath and allowed to cool to room temperature. The concentrates were quantitatively transferred to a 10 mL centrifuge tube and diluted to a 6 mL final volume by addition of methylene chloride. Alternatively, a 10 mL concentrator tube charged with 6 mL of the same stock solution was placed on the nitrogen blowdown apparatus operated at 35°C and the solution was concentrated to an apparent volume of 0.5 mL. The concentrates were quantitatively transferred to a 10 mL centrifuge tube and diluted to a 6 mL final volume by addition of methylene chloride. Both concentration procedures were done in triplicate. The resulting solutions were analyzed by GC/FID (capillary DB-5) and compared to analogous analysis of the stock solution. A separate stock solution was prepared containing compounds 17, 19, 23, 26, 27, 32, 38, 41, 43, 68, 74, and 83, and the experiment was repeated.

	EXTRACTION TYPE	EXTRACTION SEQUENCE	SOLVENT EXCHANGE
A	Continuous	1) Aqueous layer made basic, then extracted 2) Aqueous layer made acidic, then extracted	Fresh CH_2Cl_2 used for both extractions
B	Continuous	Same as A	No solvent exchange after first extraction
C	Continuous	1) Aqueous layer made acidic, then extracted 2) Aqueous layer made basic, then extracted	Fresh CH_2Cl_2 used for both extractions
D	Continuous	Same as C	No solvent change after first extraction
E	Separatory Funnel	Same as A	Fresh CH_2Cl_2 used for both extractions
F	Separatory Funnel	Same as C	Fresh CH_2Cl_2 used for both extractions

Figure 1. Experimental designs for the 6 extraction experiments.

Results and Discussion

The goal of the experiment described below and outlined in Figure 1 was to determine the most efficient sequence of operations associated with continuous liquid-liquid extractions that would maximize recovery of the compounds of interest. Any modifications which could make this procedure more economical in terms of cost, materials, or labor would be a valuable aid to the program. Specifically, this research sought to address 4 considerations: (*1*) measurement of the component recovery after acidifying the aqueous matrix and extracting, and then making basic and re-extracting (i.e., the reverse of the standard procedure); (*2*) determination of the effect of extracting the aqueous matrix at one pH and then at the second pH without changing the extracting solvent when using the continuous extraction procedure; (*3*) measurement of differences in recovery observed when the continuous extraction procedure vs the separatory funnel procedure is used; and (*4*) comparison of the micro-Snyder concentration technique vs the nitrogen blowdown concentration technique.

To answer these questions, a series of experiments were performed in which all semi-volatile TCL compounds, including organochlorine pesticides, and surrogates were spiked into an aqueous matrix and then extracted using different extraction procedures. The experimental design used in this study is summarized in Figure 1. Experiments A through D refer to the continuous liquid-liquid extraction technique. These experiments differed in the order in which the aqueous layer is acidified and made basic (A vs C) and whether or not the methylene chloride in the distilling flask was replaced following the second pH adjustment (A vs B and C vs D). Experiments E and F provide information on the efficiency of the separatory funnel technique for both pH sequences (extracting a basic layer first, E, and extracting an acidic layer first, F) and these were compared to the analo-

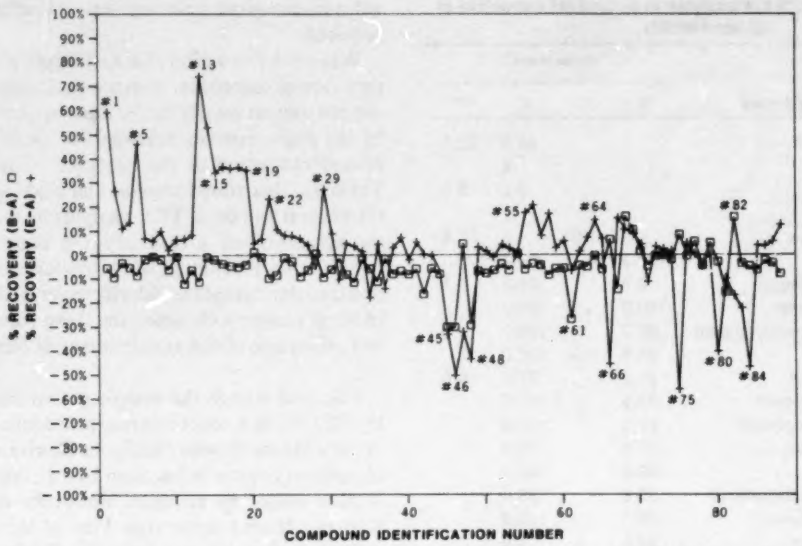

Figure 2. Change in recovery for all analytes obtained by procedures B and E relative to procedure A.

gous continuous liquid-liquid extractions. In addition, to measure which analytes were recovered by extraction of the basic layer and which were recovered by extraction of the acidic layer, the recovery for all analytes was measured for each extract prior to combining them as required by SOW. This was accomplished for experiments A, C, E, and F.

The average percent recovery and standard deviations for all semi-volatile TCL analytes analyzed in experiments A–F are presented in Table 1. The TCL analytes are numbered in groups according to the multicomponent spiking solution from which they were made (see *Experimental*). The percent recovery values for each analyte were determined by GC/MS quantification of each analyte spiked into the water matrix as described in SOW and represent the overall method efficiency.

The major emphasis of this study was on the evaluation and measurement of the differences in recovery between the various extraction methods. To reduce apparent variations in the measured recovery which may have been due to the preparation of the spiking solution, the same spiking solution was used for all samples. The differences in recovery obtained by procedures B, C, and E relative to A are shown graphically in Figures 2 and 3. The component recovery obtained by procedure A (the standard CLP procedure) is subtracted from the component recovery obtained in each experimental modification B, C, and E, and this difference is plotted for each compound in Figures 2 and 3. An analyte having a higher recovery by one of the alternative procedures will then show a positive deflection in these figures. Each experiment set was run in triplicate and analyzed within QC criteria specified in SOW. Figure 2 (B minus A) shows that procedures A and B yield essentially equivalent recoveries for all analytes except 3 of the anilines (TCL No. 45, 46, and 48) and endrin ketone (TCL No. 61). We concluded from this result that it is not necessary to change the methylene chloride in the distilling flask when the pH is adjusted, although a

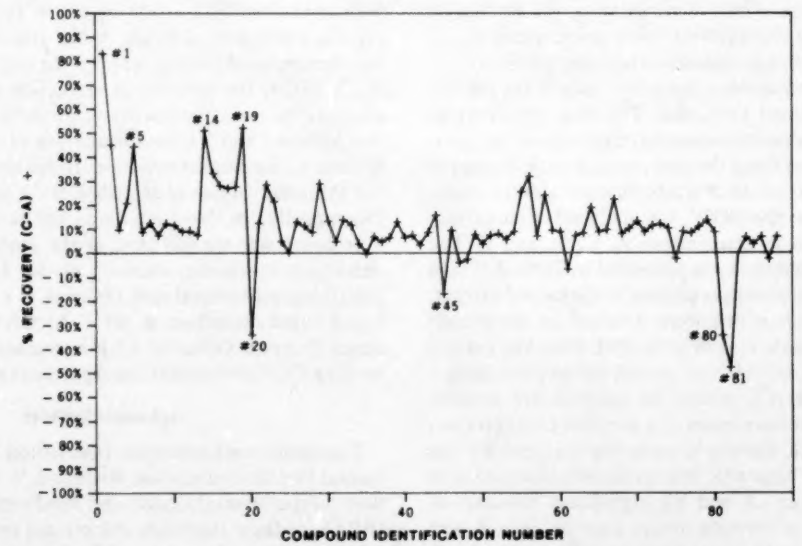

Figure 3. Change in recovery for all analytes obtained by procedure C relative to procedure A.

Table 3. Recovery (%) of analytes in second pH extraction of aqueous matrix

Cmpd ID	Compound name	Experiment A	C	E	F
28	Benzyl alcohol			28.8	22.0
45	4-Nitroaniline			1.4	
46	3-Nitroaniline			0.2	3.0
47	2-Nitroaniline				
48	4-Chloroaniline		1.8	1.8	81.6
66	Benzoic acid	100.0		94.7	
67	2,4-Dimethylphenol	0.3		82.3	0.1
68	2,4-Dinitrophenol	100.0		100.0	
69	4,6-Dinitro-2-methylphenol	99.8		100.0	
70	2-Nitrophenol	95.9	0.2	100.0	
71	4-Methylphenol	23.2		97.0	0.8
72	2,4-Dichlorophenol	93.6		100.0	
73	2,4,5-Trichlorophenol	97.6		100.0	
74	2-Chlorophenol	90.4		99.9	
75	4-Nitrophenol	100.0		100.0	
76	4-Chloro-3-methylphenol	30.8		98.4	
77	Pentachlorophenol	99.1		100.0	
78	2-Methylphenol	13.0		95.8	0.7
79	2,4,6-Trichlorophenol	98.8		100.0	
80	Phenol	74.0	1.0	92.1	11.3
81	*N*-Nitrosodiphenylamine			6.0	
83	2-Fluorophenol	97.1	0.1	100.0	0.2
84	D5-Phenol	77.0	0.2	99.2	0.3

somewhat lower recovery for anilines may occur.

Figure 2 also presents a comparison between the recoveries obtained using the continuous extraction technique vs the separatory funnel technique (E minus A). This comparison shows that although relatively volatile compounds (TCL No. 15–18) were recovered more efficiently by the separatory funnel technique, other analytes were not. Later we were able to show that the reduced recovery observed for the more volatile analytes may have been due to losses occurring during the concentration step of the procedure. We concluded from this and similar comparisons between the separatory funnel technique (E and F) and the continuous liquid-liquid technique (A–D) that the continuous liquid-liquid procedure is superior to the separatory funnel procedure for the simultaneous extraction of the TCL analytes.

Figure 3 shows the effect of performing the extraction after first acidifying the aqueous layer (experiment C). A major effect of this change appears to be the improved recovery for several of the reactive compounds such as the phthalates and the chlorinated pesticides. The base sensitivity of these compounds has been demonstrated previously (22, 24).

In addition to quantifying the analytes after combining the extracts from the extraction of the basic layer and the acidic layer as described in the SOW, we analyzed each extract prior to combination in experiments A, C, E, and F. The results from this experiment are presented in Table 3, which lists the percent of each analyte present in the second extract; only those compounds which were detected in the second extract are listed. These results show that when the extraction of the aqueous matrix is first carried out at pH 2 using a continuous extractor (C), almost no analytes are present; those that are present are present at a very low concentration in the second extract, thereby eliminating the need for the second extraction at basic pH. When the initial extraction is done on a basic layer (A and E), significant amounts of compounds capable of forming anions such as phenols and benzoic acid remain in the aqueous layers; consequently, to achieve complete recovery, extraction at the acidic pH is required.

When the extraction of a basic layer is done by the separatory funnel technique, compounds capable of forming salts remain almost totally in the aqueous layer as demonstrated by the high recovery obtained for these compounds by the second extraction of the acidified matrix (Experiment E, Table 3). This allows phenols and acids to be separated from the neutral and basic TCL analytes by this procedure. When the extraction of a basic layer is done by the continuous liquid-liquid technique, small amounts of analytes are carried into the methylene chloride layer as demonstrated by the reduced recovery obtained for those compounds by the second extraction of the acidified matrix (Experiment A, Table 3).

The final step in the sample preparation prior to analysis by GC/MS is a concentration procedure. SOW requires the use of a macro-Snyder/Kuderna-Danish apparatus to reduce the solvent volume to less than 10 mL; the final concentration is done either by nitrogen blowdown or a micro-Snyder/Kuderna-Danish apparatus. Loss of the more volatile compounds during this step is likely. Goldberg et al. (25) have demonstrated that as much as one-half of the absolute amount of the originally extracted solute can be lost during this step. Since the relative concentration actually increases during this procedure, the overall detection limit is still lowered.

These above cited losses and the experimental procedures used for these concentrations have been studied extensively by others (26–30). To determine the extent of our losses during the concentration procedure, an experiment was performed in which stock solutions of methylene chloride containing several representative TCL semi-volatiles were prepared and then concentrated by the 2 techniques described in the Statement-of-Work (see *Experimental*). The results of these experiments are presented in Table 2 and demonstrate that concentration of the sample without significant loss of analytes can be achieved by either technique.

Conclusion

The recoveries measured in the various procedures reported here demonstrate the following: (*1*) compounds which are reactive toward base are recovered more efficiently when the extraction of the aqueous matrix is done at an acidic pH first with none of the TCLs showing poorer recoveries; (*2*) replacing the methylene chloride in the distilling flask after pH adjustment is not necessary; (*3*) if the extraction is done at an acidic pH by the continuous extraction procedure, it is not necessary to make the aqueous layer basic and re-extract; (*4*) conclusions 2 and 3 allow the analysis of all TCL analytes to be done with a single extraction. If this single extract is taken to 1 mL final volume as described in the Statement-of-Work, theoretically, the detection limits will be halved.

In conclusion we feel that, of the procedures studied, the one which maximizes recovery of the TCL analytes while remaining economical and efficient is a single, continuous liquid-liquid extraction at pH 2. At this time the EPA National Program Office of CLP is evaluating the benefits of revising CLP protocols to incorporate these procedures.

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REFERENCES

(1) Comprehensive Environmental Response, Compensation, and Liability Act 42 U.S.C. && 9601-9675. As amended by the Superfund Amendments and Reauthorization Act of 1986
(2) U.S. EPA Contract Laboratory Program; Invitation for Bid WA-87K236/WA-87K237/WA 87K238. Statement-of-Work for Organic Analysis, Multi-Media, Multi-Concentration 7/87
(3) Hertz, H. S., May, W. E., Wise, S. A., & Chester, S. N. (1978) *Anal. Chem.* **50,** 429
(4) Peters, T. L. (1982) *Anal. Chem.* **54,** 1913
(5) Griffin, G. F., & Delwiche, E. A. (1982) *Anal. Chem.* **54,** 2616
(6) Matusiewicz, H. (1984) *Anal. Chem.* **54,** 1909
(7) Bhuchar, V. M., & Agrawal, A. K. (1975) *Anal. Chem.* **47,** 360
(8) Bhuchar, V. M., & Agrawal, A. K. (1981) *Analyst* **106,** 620
(9) Baker, R. J., & Suffet, I. M. (1987) in *Organic Pollutants in Water Sampling, Analysis and Toxicity Testing,* Advan. Chem. Ser. 214, I. H. Suffet & M. Malaiyandi (Eds), American Chemical Society, Washington, DC
(10) Agneessens, R., Zenon-Roland, L., & Nangniot, P. (1984) *J. High Resolut. Chromatogr. Chromatogr. Commun.* **7,** 533
(11) Murray, D. A. J. (1979) *J. Chromatogr.* **177,** 135
(12) Burgasser, A. J., & Coloruoto, J. F. (1979) *Anal. Chem.* **51,** 1588
(13) "Guidelines Establishing Test Procedures for the Analysis of Pollutants Under the Clean Water Act; Final Rule and Interim Final and Proposed Rule," (1984) 40 CFR Part 136, *Fed. Regist.* October 26, **49,** No. 209, Part VIII, 43234-43442
(14) Fisk, J. M., Haeberer, A. M., & Kovell, S. P. (1986) *Spectra* **10**(4), 22
(15) Fisk, J. F. (1986) in *Quality Control in Remedial Site Investigation Hazardous and Industrial Solid Waste Testing,* Vol. 5, ASTM STP 925, C. L. Perket (Ed.), American Society for Testing and Materials, Philadelphia, PA, p. 143
(16) Wolff, J. S., Homsher, M. T., Flotard, R. D., & Pearson, J. G. (1986) in Quality Control in Remedial Site Investigation Hazardous and Industrial Solid Waste Testing, Vol. 5, ASTM STP 925, C. L. Perket (Ed.), American Society for Testing and Materials, Philadelphia, PA, p. 157
(17) Colgrove, S. G., & Svec, H. J. (1981) *Anal. Chem.* **53,** 1737, and references therein
(18) Pellizzari, E. D., et al. (1985) Master Analytical Scheme for Organic Compounds in Water. Part I: Protocols, U.S. Environmental Protection Agency, Athens, GA, EPA-600/4-84-010a
(19) Eichelberger, J. W., Kerns, E. H., Olynyk, P., & Budde, W. L. (1983) *Anal. Chem.* **55,** 1471
(20) Sauter, A. D., Betowski, L. D., Smith, T. R., Strickler, V. A., Beimer, R. G., & Colby, B. N. (1981) *J. High Resolut. Chromatogr. Chromatogr. Commun.* **4,** 366
(21) Sauter, A. D., Betowski, L. D., & Hyatt, M. (1980) Proceedings of the 28th Annual Meeting of the American Society of Mass Spectrometry and Allied Topics, p. 169
(22) Sauter, A. D., Mills, P. E., Fitch, W. L., & Dyer (1982) *J. High Resolut. Chromatogr. Chromatogr. Commun.* **1,** 27
(23) Sauter, A. D., Betowski, L. D., & Ballard J. M. (1983) *Anal. Chem.* **55,** 116
(24) Slayton, J. L., & Trovato, E. R. (1988) EPA Report No. 903-9-88-001, U.S. Environmental Protection Agency, Washington, DC
(25) Goldberg, M. C., Delong, D., & Sinclair, M. (1973) *Anal. Chem.* **45,** 89
(26) Karasekp, F. W., Clement, R. E., & Sweetman, J. A. (1981) *Anal. Chem.* **53,** 1050A, and references therein
(27) Piet, G. J., Luijten, J. A., & Wegman, R. C. C. (1987) in *Organic Pollutants in Water Sampling, Analysis and Toxicity Testing,* Advan. Chem. Ser. 214, I. H. Suffet & M. Malaiyandi (Eds), American Chemical Society, Washington, DC
(28) Goldberg, M. C., & Weiner, E. R. (1980) *Anal Chim Acta* **115,** 373
(29) Jolley, R. L., & Suffet, I. H. (1987) in *Organic Pollutants in Water Sampling, Analysis and Toxicity Testing,* Advan. Chem. Ser. 214, I. H. Suffet & M. Malaiyandi (Eds), American Chemical Society, Washington, DC
(30) Poole, C. F., & Schuette, S. A. (1983) *J. High Resolut. Chromatogr. Chromatogr. Commun.* **6,** 526

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